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# Molecular Evidence of *Bartonella* spp. in Questing Adult *Ixodes pacificus* Ticks in California

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**Ticks are the vectors of many zoonotic diseases in the United States, including Lyme disease, human monocytic and granulocytic ehrlichioses, and Rocky Mountain spotted fever. Most known *Bartonella* species are arthropod borne. Therefore, it is important to determine if some *Bartonella* species, which are emerging pathogens, could be carried or transmitted by ticks. In this study, adult *Ixodes pacificus* ticks were collected by flagging vegetation in three sites in Santa Clara County, Calif. PCR-restriction fragment length polymorphism and partial sequencing of 273 bp of the *gltA* gene were applied for *Bartonella* identification. Twenty-nine (19.2%) of 151 individually tested ticks were PCR positive for *Bartonella*. Male ticks were more likely to be infected with *Bartonella* than female ticks (26 versus 12%, $P = 0.05$). None of the nine ticks collected at Baird Ranch was PCR positive for *Bartonella*. However, 7 (50%) of 14 ticks from Red Fern Ranch and 22 (17%) of 128 ticks from the Windy Hill Open Space Reserve were infected with *Bartonella*. In these infected ticks, molecular analysis showed a variety of *Bartonella* strains, which were closely related to a cattle *Bartonella* strain and to several known human-pathogenic *Bartonella* species and subspecies: *Bartonella henselae, B. quintana, B. washoensis,* and *B. vinsonii* subsp. *berkhoffii*. These findings indicate that *I. pacificus* ticks may play an important role in *Bartonella* transmission among animals and humans.**

*Bartonella* spp. are emerging pathogens, as new *Bartonella* species have been identified in humans and a wide range of mammals in recent years (5, 17, 18, 20–23, 27, 28; B. B. Chomel, R. W. Kasten, C. C. Chang, K. Yamamoto, R. Heller, S. Maruyama, H. Ueno, D. Simpson, S. S. Swift, Y. Piemont, and N. C. Pedersen, Abstr. Int. Conf. Emerg. Infect. Dis., p. 21.10, 1998; R. Heller, M. Kubina, G. Delacour, I. Mahoudeau, F. Lamarque, M. Artois, H. Monteil, B. Jaulhac, and Y. Piemont, Abstr. 97th Gen. Meet. Am. Soc. Microbiol., abstr. B-505, p. 115, 1997; R. Heller, M. Kubina, G. Delacour, F. Lamarque, G. Van Laere, R. Kasten, B. Chomel, and Y. Piemont, Abstr. Int. Conf. Emerg. Infect. Dis., p. 21.18, 1998). Several *Bartonella* species and subspecies are important human pathogens that cause a variety of clinical syndromes, and most *Bartonella* organisms are arthropod borne. *Bartonella bacilliformis* is the agent of Carrión's disease, which is mainly found in the Andes mountains, and is transmitted by sand flies (3). *B. bacilliformis* infection is characterized by a biphasic process. The acute form of the infection, Oroya fever, causes severe and life-threatening hemolytic anemia, and the chronic form, verruga peruana, results in vascular proliferative lesions of the skin (12). *B. quintana,* the agent of trench fever, is transmitted by the human body louse (36). *B. quintana* was also identified as one of two agents causing bacillary angiomatosis (26). In urban centers, human cases of this infection were recently observed in homeless people and alcohol abusers. Cat scratch disease (CSD), caused by *B. henselae,* is an important zoonosis with cats serving as the major reservoir (25) and cat fleas (*Ctenocephalides felis*) as the vector (16). Although CSD is usually a self-limiting disease in immunocompetent patients, atypical forms of this infection can occur, such as Parinaud's oculoglandular syndrome, encephalopathy, osteomyelitis, or endocarditis (1). In immunocompromised patients, *B. henselae* is the other causative agent of bacillary angiomatosis (40). *B. vinsonii* subsp. *berkhoffii* infection was reported in several canine endocarditis cases (7, 10) and recently in one human endocarditis case (41). Based on a seroepidemiological study (38), *B. vinsonii* subsp. *berkhoffii* infection has been suggested to be tick transmitted. Recently, new human pathogenic *Bartonella* strains have been identified, but their vectors remain unknown. For example, *B. grahamii* was associated with one human case of neuroretinitis (24), but the mode of transmission was not identified. A human patient with fever and neurological signs was infected with a novel strain, *B. vinsonii* subsp. *arupensis,* possibly from a rodent source (22, 45). Furthermore, in Washoe County, Nev., a human case of myocarditis was related to a new *Bartonella* organism, *B. washoensis*. Rodents were determined to be the likely reservoir (9).

Ticks are the vectors of many zoonotic diseases in the United States, including Lyme disease, human monocytic and granulocytic ehrlichioses, and Rocky Mountain spotted fever. Various *Bartonella* species have been isolated from small rodents (4, 5, 17, 18, 20–22, 28). Natural coinfection of *Bartonella* species and *Borrelia burgdorferi* has also been demonstrated in wild-caught mice (22). Because these small mammals usually serve as a blood source for larval and nymphal ticks, it is of great interest to determine if certain *Bartonella* organisms could be tick borne. In order to investigate the possible transmission of *Bartonella* organisms by ticks, it is important to first demonstrate the presence of *Bartonella* in questing adult ticks from a natural environment. Therefore, the objective of this study was to determine if *Bartonella* organisms could be detected in questing adult ticks collected by flagging vegetation.

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The tick species carrying *Bartonella* is likely to be *I. pacificus,* since it is a very common tick species in California and the known vector for *Borrelia burgdorferi*. As a follow-up to previous epidemiological studies of *Bartonella* in coyotes from California (13, 15), *I. pacificus* ticks were collected from Santa Clara County, Calif., where *Bartonella* infection is enzootic in coyotes.

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## MATERIALS AND METHODS

**Tick collection.** The sample size ($n$) of ticks to be tested in this study was calculated based on the following formula: $(1 - \text{prevalence of infection in ticks})^n = (1 - \text{percentage of confidence})$. It was assumed that the prevalence of *Bartonella* in ticks from this area should be low, as the prevalence for *Borrelia burgdorferi* infection in *I. pacificus* from California has been reported to be approximately 2% (29, 32). Therefore, the collection of 150 ticks was required for a 95% confidence of finding at least one *Bartonella*-positive tick. A total of 151 *I. pacificus* adult ticks was captured by flagging vegetation from areas in Santa Clara County, Calif.: Red Fern Ranch (14 ticks), Baird Ranch (9 ticks), and Windy Hill Open Space Reserve (128 ticks). Collection and identification of tick species were carried out by the Vector Control Section, Wildlife Unit, Santa Clara County Department of Health Services, from December 1998 to January 1999. After collection, ticks were stored at −70°C until DNA extraction was performed.

**DNA extraction from ticks.** DNA extraction using the DNeasy tissue kit (Qiagen, Hilden, Germany) was performed by following the manufacturer's instructions, with minor modifications. Before extraction, ticks were individually put in 1.5-ml microtubes containing 1 ml of 70% ethanol solution for 1 min and briefly rinsed with sterile water. Each tick was then transferred to a clean microtube and macerated in 180 μl of Buffer ATL (Qiagen). After addition of 20 μl of proteinase K (18 mg/ml) (Qiagen), samples were incubated in a 50°C water bath overnight for complete lysis of soft tissues inside the ticks. The lysed samples were treated with 20 μl of RNase A (10 mg/ml) for 2 min at room temperature and then incubated at 70°C for 10 min after addition of 200 μl of Buffer AL (Qiagen). To inactivate potential infectious agents, samples were then heated at 95°C for 15 min. The following steps of DNA extraction were performed according to the manufacturer's instructions.

**PCR-RFLP procedures.** PCR amplification was performed with *Bartonella*-specific primers of the citrate synthase (*gltA*) gene as previously described (14). Undiluted DNA and a 1:5 dilution of the extracted DNA from each macerated tick were used as DNA templates. The PCR-amplified products were verified by gel electrophoresis for the appearance of an approximately 400-bp fragment of the *gltA* gene. If extra bands were observed, further purification of the 400-bp band of the *gltA* gene was conducted. A small piece of the agarose at the position of 400 bp was cut from the agarose gel with a sterile tip through UV-light visualization, mixed with 50 μl of sterile water in a 1.5-ml microtube, and melted at 100°C for 10 min. The final product, kept at 50°C, was used as the template for a secondary PCR. *B. vinsonii* subsp. *berkhoffii* DNA was added to a *Bartonella* PCR-negative *I. pacificus* extract as a positive control. A negative control was made by using sterile water instead of template DNA. In order to prevent laboratory contamination, different isolated areas were used for DNA extraction and PCR preparation. Disposable sterile vials and filter tips were used for DNA extraction and PCR reagent preparation. The PCR chamber was used only for the preparation of PCR reagents and not for the isolation or subculture of *Bartonella* spp. To minimize bacterial contamination in the laboratory, all isolations were performed in a safety class II hood cabinet. The positive control of *B. vinsonii* subsp. *berkhoffii* was systematically prepared last, to prevent possible cross contamination with the tested samples. The final purified PCR product of the *gltA* gene was then used for PCR-restriction fragment length polymorphism (PCR-RFLP) and further sequencing analyses. *Taq*I (Promega, Madison, Wis.), *Hha*I (New England Biolabs, Beverly, Mass.), and *Mse*I (New England Biolabs) restriction endonucleases were used for PCR-RFLP analysis of the *gltA* gene. The digestion conditions used were those recommended by the enzymes' manufacturers. Banding patterns were compared to the profiles of *B. henselae* Houston-1 (ATCC 49882), *B. clarridgeiae* (ATCC 700095), *B. quintana* (ATCC VR-358), *B. bacilliformis* (ATCC 35686), *B. elizabethae* (ATCC 49927), *Bartonella* strain cattle-1 (University of California, Davis), *B. vinsonii* subsp. *vinsonii* (ATCC VR-152), and *B. vinsonii* subsp. *berkhoffii* (ATCC 51672).

TABLE 1. Epidemiological characteristics of *Bartonella* infection in *Ixodes pacificus* adult ticks from Santa Clara County

| Origin | Sex | % of ticks with *Bartonella* infection (no. PCR positive/total no. tested) |
|---|---|---|
| Baird Ranch | Male | 0 (0/4) |
| | Female | 0 (0/5) |
| Red Fern Ranch | Male | 42.9 (3/7) |
| | Female | 57.1 (4/7) |
| Windy Hill Open Space Reserve | Male | 25.8 (17/66) |
| | Female | 8.1 (5/62) |
| Total | | 19.2 (29/151) |

**DNA sequencing.** All PCR-positive tick samples were sequenced for the *gltA* gene as previously described (14). First, the BLASTN program of the GCG software (Wisconsin Sequence Analysis Package, version 10; Genetics Computer Group) was applied to determine the bacterial species and subspecies closest to the sequencing results of the *Bartonella*-positive tick samples, based on the DNA sequence similarity of 273 bp of the *gltA* gene. Then, the GAP program was used for sequence alignments and determination of the percentage of DNA similarity between the sequences of the *gltA* gene for a *Bartonella*-positive tick sample and the closest bacterial species and subspecies. The EMBL-GenBank accession numbers for the citrate synthase gene sequences of the strains used for the sequence comparisons were as follows: *B. vinsonii* subsp. *berkhoffii,* U28075; *B. henselae* Houston-1, L38987; *B. quintana,* Z70014; *B. washoensis,* AF050108; and *Bartonella* strain cattle-1, AF228768.

**Statistical analysis.** The data were tabulated by SAS, version 6.12, and then analyzed by Epi-Info, version 6.03. The chi-square test for homogeneity was used to evaluate the association between the infection and a categorized factor (i.e., gender of ticks), and $P$ values were calculated with the Yates corrected method.

## RESULTS

Twenty-nine (19.2%) of 151 individually processed adult ticks tested were PCR positive for *Bartonella* (Table 1). None of the ticks collected from Baird Ranch was PCR positive for *Bartonella*. However, 7 (50%) of 14 ticks from Red Fern Ranch and 22 (17%) of 128 ticks from the Windy Hill Open Space Reserve were infected with *Bartonella*. Overall, male ticks were more likely to be infected with *Bartonella* than female ticks (26 versus 12%, $P = 0.05$). After stratification by tick collection location, an even distribution of *Bartonella* infection was observed in male and female ticks from Red Fern Ranch. However, for ticks from the Windy Hill Open Space Reserve, male ticks were more likely to be *Bartonella* PCR positive than female ticks ($P < 0.05$).

After PCR-RFLP analysis for *Bartonella* species identification with three different endonucleases, three ticks (ticks 4, 5, and 12) collected at Red Fern Ranch were found to be infected with a *Bartonella*-like strain, for which the digestion profile was similar to that of *B. quintana* (Table 2 and Fig. 1). The partial sequence analysis of the *gltA* gene showed that the closest related sequence was in the *B. quintana* Fuller strain, with a DNA similarity ranging from 97.8 to 100% (Table 2). The PCR-RFLP profiles of the *Bartonella*-like strains from six ticks (ticks 15, 17, 22, 34, 74, and 80) collected at the Windy Hill Open Space Reserve were identical to the profile of *B. henselae* (Table 2 and Fig. 1). The nucleotide sequences of the partial *gltA* gene of these six samples showed a 97.4 to 100.0% sequence similarity to the *gltA* sequence of the *B. henselae* Hous-

TABLE 2. PCR-RFLP and partial sequencing analyses of the *gltA* gene for *Bartonella* species identification in 18 infected ticks

| Origin | Tick | PCR-RFLP profile[a] | Closest *Bartonella* species (% DNA similarity based on 273 bp of the *gltA* gene) |
|---|---|---|---|
| Red Fern Ranch | 4 | *B. quintana*-like | *B. quintana* (97.8) |
| | 5 | *B. quintana*-like | *B. quintana* (99.6) |
| | 12 | *B. quintana*-like | *B. quintana* (100.0) |
| Windy Hill Open Space Reserve | 15 | *B. henselae*-like | *B. henselae* (99.6) |
| | 17 | *B. henselae*-like | *B. henselae* (98.5) |
| | 22 | *B. henselae*-like | *B. henselae* (99.3) |
| | 34 | *B. henselae*-like | *B. henselae* (97.4) |
| | 63 | *Bartonella* strain cattle-1-like | *Bartonella* strain cattle-1 (99.6) |
| | 70 | Mixed type of *B. henselae* and *B. vinsonii* subsp. *berkhoffii* | *B. vinsonii* subsp. *berkhoffii* (98.9) |
| | 73 | *Bartonella* strain cattle-1-like | *Bartonella* strain cattle-1 (99.3) |
| | 74 | *B. henselae*-like | *B. henselae* (98.9) |
| | 75 | Mixed type of *B. henselae* and *Bartonella* cattle-1 strain | *Bartonella* strain cattle-1 (99.6) |
| | 80 | *B. henselae*-like | *B. henselae* (100.0) |
| | 81 | *Bartonella* strain cattle-1-like | *Bartonella* strain cattle-1 (99.6) |
| | 93 | *Bartonella* strain cattle-1-like | *Bartonella* strain cattle-1 (99.3) |
| | 143 | Unrecognized type | *B. washoensis* (99.3) |
| | 146 | Unrecognized type | *B. washoensis* (100.0) |
| | 147 | Unrecognized type | *B. washoensis* (100.0) |

[a] PCR-RFLP profile of *B. quintana*: 310-bp band with *Taq*I digestion, 400-bp band with *Hha*I digestion, and 150- and 200-bp bands after *Mse*I digestion. PCR-RFLP profile of *B. henselae*: 90-, 150-, and 190-bp bands with *Taq*I digestion, 190- and 220-bp bands with *Hha*I digestion, and 150- and 200-bp bands with *Mse*I digestion. PCR-RFLP profile of *Bartonella* strain cattle-1: 150-and 250-bp bands with *Taq*I digestion, 400-bp band with *Hha*I digestion, and 140- and 200-bp bands with *Mse*I digestion. PCR-RFLP profile of the mixed type of *B. henselae* and *B. vinsonii* subsp. *berkhoffii*: 90-, 150-, and 190-bp bands with *Taq*I digestion, 190-, 220-, and 400-bp bands with *Hha*I digestion, and 80-, 150-, 200-, and 210-bp bands with *Mse*I digestion. PCR-RFLP profile of the mixed type of *B. henselae* and *Bartonella* strain cattle-1: 90-, 150-, 190-, and 250-bp bands with *Taq*I digestion, 190-, 220-, and 400-bp bands with *Hha*I digestion, and 140- and 200-bp bands with *Mse*I digestion.

ton-1 strain (Table 2). A profile similar to that of a cattle *Bartonella* strain (13) was identified in five ticks (ticks 63, 73, 75, 81, and 93) collected at the Windy Hill Open Space Reserve, including one tick (tick 75) with a mixed profile of *B. henselae* and *Bartonella* cattle-1 strains (Table 2 and Fig. 1). All of these strains showed very high percentages of DNA similarity compared to the cattle *Bartonella* strain, ranging from 99.3 to 99.6% (Table 2). One tick (tick 70) was infected with a *Bartonella*-like strain closely related to *B. vinsonii* subsp. *berkhoffii,* based on a 98.9% nucleotide similarity by partial sequence analysis of the *gltA* gene (Table 2). However, the PCR-RFLP profile of this tick extract showed a mixed PCR-RFLP profile of *B. vinsonii* subsp. *berkhoffii* and *B. henselae* (Table 2 and Fig. 1). There were three ticks (ticks 143, 146, and 147) infected with *Bartonella*-like strains with previously unrecognized PCR-RFLP profiles (Table 2 and Fig. 1) compared to the available *Bartonella* type strains tested. After sequence comparisons of the partial *gltA* gene, these strains were determined to be closely related to *B. washoensis* (DNA similarity from 99.3 to 100.0%) (Table 2). Based on the partial sequences of the *gltA* gene, all strains obtained from the PCR-positive ticks were significantly different from *Rickettsia prowazekii* (DNA similarity of <70%), a species closely related to *Bartonella* spp., and from *Coxiella burnetii* (DNA similarity of <70%). In the 11 remaining *Bartonella*-PCR positive ticks, despite a PCR-RFLP profile suggestive of *Bartonella* coinfection, partial sequencing analysis was inconclusive.

## DISCUSSION

To demonstrate the role of ticks as vectors for *Bartonella* in natural environments, questing ticks have to be examined. This

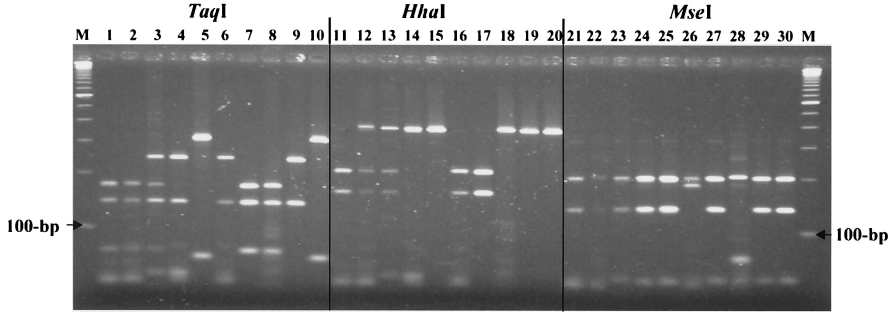


FIG. 1. PCR-RFLP of the *gltA* gene of tick samples. Assays were performed by digestion with *Taq*I, *Hha*I, and *Mse*I. Lane M, 100-bp molecular size ladder; lanes 1, 11, and 21, tick 22; lanes 2, 12, and 22, tick 70; lanes 3, 13, and 23, tick 75; lanes 4, 14, and 24, tick 81; lanes 5, 15, and 25, tick 12; lanes 6, 16, and 26, tick 143; lanes 7, 17, and 27, *B. henselae*; lanes 8, 18, and 28, *B. vinsonii* subsp. *berkhoffii*; lanes 9, 19, and 29, *Bartonella* strain cattle-1; lanes 10, 20, and 30, *B. quintana*.

approach has been successfully used for Lyme borreliosis and the agents of human ehrlichioses in *I. pacificus* ticks in western North America (29, 30, 32). The *gltA* gene was chosen for PCR-RFLP and partial sequencing analysis because this gene is more variable, and thus more discriminative for identifying *Bartonella* species and subspecies, than the more conserved 16S rRNA gene (6, 42). To our knowledge, this is the first report of *Bartonella* infections identified in questing *I. pacificus* adult ticks. Not only was a high prevalence (19.2%) of *Bartonella* infection observed in these ticks, but also partial sequences of several previously recognized human *Bartonella* pathogens, i.e., *B. quintana, B. henselae, B. washoensis,* and *B. vinsonii* subsp. *berkhoffii,* were also identified in these infected ticks. These results indicate that these human-pathogenic *Bartonella* species or closely related species could be carried by *I. pacificus* ticks. However, it is unclear why male ticks were more likely to be PCR positive for *Bartonella* than females. This finding will require further confirmation with a larger sample size of male and female ticks from various geographical areas.

Based on previous studies, a significant percentage of human *Bartonella* infections have occurred without exposure to known reservoirs or vectors. Up to 30% of human CSD patients may not have been scratched or bitten by cats (33), and 1% of human CSD patients do not have any known contact with animals (35). Although fleas are the main vectors for *B. henselae* among cats (16), ticks have been suspected to be the source of infection in a few human cases of *B. henselae* infection (34). An epidemiological study conducted by Zangwill et al. (46) showed that CSD patients were more likely to have found one tick on their bodies than were controls. In a recent outbreak of trench fever in Seattle, Wash., vectors other than lice were suspected in *B. quintana* transmission because no association was found between body louse infestation and the infection in these human patients (44). In the southeastern United States, two cases of *B. quintana*-associated central nervous system infection were reported for children with no known history of louse exposure, including one case of rural origin (39). Furthermore, *Dermacentor andersoni* ticks were identified as competent vectors for *B. bacilliformis* in an experimental infection of nonhuman primates (37), and a *B. bacilliformis*-like agent was recently isolated from an *I. ricinus* tick in Wa̜lcz, Poland (31). According to a recent seroepidemiological study of dogs from North Carolina and Virginia, heavy tick infestation was a more important factor associated with *B. vinsonii* subsp. *berkhoffii* infection than heavy flea infestation (38). Breitschwerdt et al. further found that dogs infected with tick-transmitted *Ehrlichia* species are frequently coinfected with *B. vinsonii* subsp. *berkhoffii* (8). These findings suggest that ticks could play a role in the transmission of *Bartonella* organisms.

*I. pacificus* is very common in California, as it has been identified in 50 of the 58 counties (19). These ticks are three-host *Ixodid* ticks. During their larval and nymphal stages, they feed mainly on small mammals and reptiles (19). As rodents are the likely reservoir for *B. washoensis* (9), a *Bartonella* species isolated from a patient with myocarditis (R. L. Regnery et al., unpublished data), these small mammals could serve as an important source of infection to *Ixodes* larvae and nymphs. If transstadial transmission was successful after molting, questing *I. pacificus* nymphal or adult ticks could then transmit *B. washoensis* to large animals and possibly humans through tick bites.

The likelihood of ticks acquiring *B. henselae* and *B. vinsonii* subsp. *berkhoffii* from rodents should be low, as these *Bartonella* organisms have not yet been identified in any rodent reservoir (4, 5, 17, 18, 20–22, 28). Nevertheless, as *B. vinsonii* subsp. *vinsonii* and *B. vinsonii* subsp. *arupensis* have been identified in rodents (2, 22, 45), the existence of a rodent reservoir for *B. vinsonii* subsp. *berkhoffii* still needs to be further investigated. Regarding other sources of infection, there is evidence that cats are the main reservoir for *B. henselae,* and *B. vinsonii* subsp. *berkhoffii* has been isolated from domestic dogs and a large number of California wild coyotes (*Canis latrans*) (7, 8, 10, 13, 15, 27). Although these large mammals are not the preferred hosts for *I. pacificus* nymphs, their role as accidental hosts for the nymphs cannot be excluded (19). However, the percentage (3% [5 of 151]) of *Bartonella* PCR-positive ticks that were found to be infected with a *B. henselae*-like strain could not be explained simply by this hypothesis and would suggest the possibility of other reservoirs. In the present study, several ticks were demonstrated to be infected with *B. henselae*-like strains, supporting previous studies involving ticks as a possible source of human infection (34, 46). Similarly, the identification of *B. vinsonii* subsp. *berkhoffii* in one questing *I. pacificus* adult tick supports the potential role of tick transmission, as previously suggested for domestic dogs (38). Furthermore, the major foci of *B. vinsonii* subsp. *berkhoffii* infection in coyotes were distributed mainly in California's coastal and central counties, which coincides with the known distribution of several arthropods, including *I. pacificus* ticks (13, 15).

The identification of the *B. quintana*-like strain in *I. pacificus* ticks is quite surprising and raises questions about the role of this arthropod in human infection. At present, humans are considered to be the only reservoir for *B. quintana*. The likelihood that ticks acquire *B. quintana* directly from humans must be considered very low, as humans are not the preferred host for *I. pacificus* nymphs. Moreover, it would be necessary for a nymphal tick to feed on a *B. quintana*-bacteremic human long enough to acquire the infection. Our results could suggest the presence of other reservoirs for *B. quintana,* which could serve as an alternative blood source of infection to *I. pacificus* nymphs. Western fence lizards (*Sceloporus occidentalis*) have been found to be one of the preferred hosts for immature *Ixodes* ticks in California (19), but no investigation has been conducted to isolate *Bartonella* spp. in these vertebrates. Therefore, attempts to isolate various *Bartonella* species from lizards will be of major importance.

In California, high *Bartonella* bacteremia prevalences have been reported for beef cattle (89%) and mule deer (90%), and ticks have been suspected to be a potential source of infection for these ruminants based on epidemiological evidence (13). Similarly, 98% of 58 roe deer (*Capreolus capreolus*) from France were *Bartonella* bacteremic (Heller et al., Abstr. Int. Conf. Emerg. Infect. Dis., p. 21.18) and 60% of 121 tick samples collected on roe deer in The Netherlands reacted with a *Bartonella* genus-specific probe (43). However, such a high percentage of positive ticks must be interpreted with caution, as most ticks could have fed on bacteremic animals. On the contrary, we detected *Bartonella* infection in questing adult ticks, suggesting infection at an earlier stage. We also identi-

fied infection of these ticks with a *Bartonella* strain similar to *Bartonella* isolated from domestic and wild ruminants and to *B. weissii,* initially isolated from domestic cats (13). Therefore, it will be necessary to evaluate the possible role of ticks in transmission between cattle and domestic cats.

It will also be important to determine the possibility of transovarial and transstadial transmission of these *Bartonella* organisms in female *I. pacificus* ticks. In the case of Rocky Mountain spotted fever, after the last meal of *D. andersoni* adult females on large mammals, *Rickettsia rickettsii* can invade the ovaries of adult female ticks and infect the oocytes, producing infected larvae (11). Despite the absence of *Bartonella* bacteremia in the preferred hosts for immature *I. pacificus* ticks, transovarial and transstadial transmissions of *Bartonella* could represent another route of infection for the questing adult ticks to acquire these *Bartonella* infections.

Based on the PCR-RFLP analysis of the *gltA* gene with three different endonucleases, two tick samples showed a mixed profile of different *Bartonella* strains, one with *B. henselae* and *Bartonella* strain cattle-1 and the other with *B. henselae* and *B. vinsonii* subsp. *berkhoffii*. The mixed profile could result from a coinfection in ticks that fed on different hosts infected with various *Bartonella* species and subspecies. Nevertheless, the partial sequences of the *gltA* gene identified only one specific *Bartonella* strain in these two samples.

To confirm the role of *Ixodes* ticks in *Bartonella* transmission, epidemiological and experimental transmission studies will be necessary. We could not demonstrate if the *Bartonella* strains identified in the tick samples were still alive and transmissible to the next host. However, such a high prevalence of *Bartonella* infection in *I. pacificus* ticks from natural environments warrants further investigation all over California to elucidate the potential role of this tick species as a vector for *Bartonella* organisms, especially for human *Bartonella* pathogens. Finally, and most importantly, clinicians need to be aware of possible *Bartonella* infections in human patients after tick bites.

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